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The effects of treatment on quality parameters of smoothie-type 'Hayward' kiwi fruit beverages

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Kiwi fruits contain high levels of bioactive compounds (ascorbic acid, total phenols, anthocyanins, chlorophylls, carotenoids, tannins, flavanols and flavonoids), which are important to preserve them in preparation of purees, beverages, juices and other varieties of food products. The purpose of the present study was to evaluate physicochemical parameters, including antioxidant and binding properties during different treatments in the preparation of 'Hayward' kiwi fruit smoothie-type beverages. Preparation of smoothie-type beverages from kiwi fruits involved the following stages: adding of different percentages of sugar content inside the mass, mixture and then freezing during long storage. The bioactive compounds were determined in fresh and treated smoothies. To assess the antioxidant capacity, four different methods were applied: 2, 2′-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$), Ferric-reducing/antioxidant power (FRAP), Cupric reducing antioxidant (CUPRAC) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays, showing high correlation coefficients with polyphenols from 0.894 till 0.969, because polyphenols mostly influence the antioxidant capacity. The decrease of total phenolic compounds (39%) and anthocyanins (31%) was higher than in ascorbic acid (25%) in preparation of smoothies after long cold storage. The proposed above preparation of smoothies preserved the decrease of bioactive compounds. The binding abilities of extracted polyphenols to human serum albumin (HSA) of smoothies determined by 3D-fluorescence and FTIR spectroscopy correlated with their amounts. The investigated kiwi fruit smoothies can be used as a source of antioxidants.

1. Introduction

The qualities of kiwi fruit as a source of antioxidants have been described in a number of articles and compared with mangosteen, mango, snake fruit and other fruits (Gorinstein et al., 2011; Leontowicz et al., 2016). Kiwi fruits have strong antioxidant effects and may prevent the development and deterioration of diseases caused by oxidative stress (Iwasawa, Morita, Yui, & Yamazaki, 2011). In the last years it became popular to introduce for consumption in addition to fresh fruits and vegetables also different minimally processed juices, beverages, purees and smoothies (Auger et al., 2011; George, Waroonphan, Niwat, Gordon, & Lovegrove, 2013; Tadapaneni, Edirisinghe, & Burton-Freeman, 2015). Sampedro, Fan, and Rodrigo (2010) showed that consumers are also increasing the demand for more convenient, nutritious, fresh and price-reasonable products. Pureed fruit and vegetable products are useful for increasing micronutrient status and plasma antioxidant capacity in order to contribute to coronary heart disease (George et al., 2013). Fruit juices, purees and smoothies were prepared with a wide variety of fruits and berries such as blackcurrant, aronia, cranberry, blueberry, lingonberry, grape, kiwi fruit, quince-enriched and apple-enriched pumpkin

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puree (Nawirska-Olszanska, Biesiada, Sokol-Letowska, & Kucharska, 2011). Kebede et al. (2015) showed that the preparation of different purees involved thermal treatment and then proposed an integrated fingerprinting for shelf-life testing of chemical changes in thermally treated carrot puree. Alvarez-Fernandez, Hornedo-Ortega, Cerezo, Troncoso, and Garcia-Parrilla (2014) studied the impact of the strawberry puree elaboration process on the chemical composition of the final products. The application of hydrostatic high pressure on an industrial line of nectarine purees was compared with the traditional thermal treatment of pasteurization (Garcia-Parra et al., 2011). Oszmianski, Wolniak, Wojdylo, and Wawer (2008) prepared Idared and Shampion apple purees and showed the changes in the polyphenols during the storage. The impact of microwave and conventional heat pasteurization and storage on total and individual carotenoids and chlorophylls and shelf-life in kiwi fruit puree was evaluated (Benlloch-Tinoco et al., 2015a, 2015b). As it was described above the purees were mostly prepared under high pressure and high temperature, and as it was mentioned previously a number of reports was connected with such ways of technological preparations. It is a lack in the present literature about the preparation of fruit smoothies, especially from kiwi fruits. We proposed preparation of smoothie-type beverages without pasteurization and high preservation of antioxidant activity under freezing. This is a simple process without any heating during the preparation of the fruits. As kiwi fruits contain high levels of polyphenols, the aim of the present study was to conserve the ability of the smoothies to have a high content of bioactive compounds.

2. Materials and methods

2.1. Sample preparation

Kiwi fruit cultivar ‘Hayward’ was grown under conventional conditions in the orchard Heanam County (longitude 126° 15″ and latitude 34° 18″), Jeonnam province, Korea. The harvest date was October 30, 2014 and October 18, 2015.

2.2. Treatment of kiwi fruit to obtain smoothies

Preparation of smoothies involved the following steps: peeling of fruits, cutting manually with plastic knife to small slices, and grounding. Then the fruits were divided to five separate parts and 0, 10, 15, 20, 25% white sugar were sprayed on kiwi fruit and placed for 2–3 h in ambient temperature in order sugar to immerse into kiwi fruits. One part was as a control (fresh smoothie without any sugar). From each part about 3 kg of kiwi fruit were put into PE film and then stored at −25 °C in deep freezer for 4 months (Fig. 1). After the storage for 4 months the smoothies were used also as a beverage source. Three replicates of kiwi fruit smoothie's preparation were carried out. After storage, the smoothies were subjected to analyses. The samples were treated with liquid nitrogen in order to prevent oxidation of phenolic compounds and then lyophilized as previously described (Gorinstein et al. 2011; Park et al. 2013 and 2014). The smoothies were done by advice of engineer Sang-Bong Choi, director of Bohae Brewery Co., Ltd. 15, Daean-dong, Mokpo-si. Jeollanam-do 530–280, South Korea, http://www.bohae.co.kr. The smoothies after storage were transported under frozen status. The smoothie or juice was done using juice maker (frozen kiwi fruit were broken as ice). The cold drink usually used in Korea even though winter season.

2.3. Chemicals

6- Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picryl-hydrazl (DPPH), 2,9-dimethyl-1,10-phenanthro line (neocuproine), Folin-Ciocalteu reagent were purchased from Sigma Chemical Co., St. Louis, MO, USA. 2,4,6,-tripyridyl-*s*-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade.

2.4. Determination of bioactive compounds and total antioxidant capacity

Polyphenols were extracted from lyophilized kiwi fruit smoothies with methanol and water (concentration 25 mg/ml) at room temperature twice during 3 h in order to find optimal extraction of polyphenols. Polyphenols were determined by Folin-Ciocalteu method with measurement at 750 nm using a spectrophotometer (Hewlett-Packard, model 8452A, Rockvile, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g DM (Singleton, Orthofer, & Lamuela-Raventos, 1999). Flavonoids, extracted with 5% $NaNO_2$, 10% $AlCl_3 \times H_2O$ and 1 M NaOH, were measured at 510 nm. Condensed tannins (procyanidins) were extracted with 4% vanillin solution in MeOH and the extracts were measured at 500 nm. The total flavanols were estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance at 640 nm was read (Arnous, Makris, & Kefalas, 2001). (+)Catechin served as a standard for flavonoids, flavanols and tannins, and the results were expressed as catechin equivalents (CE). The total anthocyanins were measured by a pH differential method, using the following equation: $A = [(A_{510}-A_{700})\ pH_{1.0}-(A_{510}-A_{700})\ pH_{4.5}]$. Results were expressed as milligrams of cyanidin-3-glucoside equivalent (CGE) per gram of DW (Cheng & Breen, 1991).

Total Antioxidant Capacity (TAC) was determined with utilization of four complementary assays in order to receive reliable results: (1) 2, 2′-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\cdot +}$) of ABTS (7 mM) and $K_2S_2O_8$ (2.45 mM). This solution was diluted with methanol until the absorbance in the samples reached 0.7 at 734 nm (Re et al., 1999). (2) Ferric-reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants in the investigated samples to reduce ferric-tripiridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}), which absorbs light at 593 nm (Benzie & Strain, 1996). (3) Scavenging free radical potentials were tested in a methanolic solution of 1, 1-diphenyl-2-picrylhydrazyl method (DPPH), with absorption band at 515 nm which disappears upon reduction by antiradical compounds. DPPH solution (3.9 ml, 25 mg/l) in methanol was mixed with the samples extracts in water or methanol (0.1 ml), then at 515 nm the absorbance was stable (Brand-Williams, Cuvelier, & Berset, 1995). (4) Cupric reducing antioxidant (CUPRAC): to the mixture of 1 ml of cooper (II) − neocuproine and NH_4Ac buffer solution, acidified and non acidified methanol extracts of fruits (or standard) solution (x, in ml) and H_2O [(1.1−x) ml] were added to 4.1 ml. The absorbance at 450 nm was recorded (Apak, Guclu, Ozyurek, & Karademir, 2004).

Total chlorophylls, chlorophylls **a and b** and total carotenoids were extracted with 100% acetone and determined spectrophotometrically at the following absorbances (nm): 661.6, 644.8 and 470, respectively (Boyer, 1990).

Total ascorbic acid was determined by CUPRAC assay in water extract (100 mg of lyophilized sample and 5 ml of water) (Ozyurek, Guclu, Bektasoglu, & Apak, 2007).

2.5. Fluorometric and FTIR measurements

Fluorometric measurements were used for the evaluation of binding properties of kiwi fruit smoothies extracts to human serum

Fig. 1. Preparation of kiwi fruit smoothies.

albumin (HSA). Two dimensional (2D-FL) and three dimensional (3D-FL) fluorescence measurements were recorded on a model FP-6500, Jasco spectrofluorometer, serial N261332, Japan, equipped with 1.0 cm quartz cells. For the fluorescence measurement, 3.0 ml of 2.0×10^{-6} mol/L HSA solution and various amounts of kiwi fruit smoothies extracts were added to a 1.0 cm quartz cell manually using a micro-injector. The corresponding fluorescence emission spectra were then recorded in the range of 300–500 nm upon excitation at 280 nm in each case. The three-dimensional fluorescence spectra were measured under the following conditions: the emission wavelength was recorded between 200 and 795 nm. All solutions for protein interaction were prepared in 0.05 mol/L Tris-HCl buffer (pH 7.4), containing 0.1 mol/L NaCl (Park et al., 2014).

The presence of polyphenols in the investigated smoothies was studied by Fourier transform infrared n (FT-IR) spectroscopy. A Nicolet iS 10 FT-IR Spectrometer (Thermo Scientific Instruments LLC, Madison, WI, USA), with the smart iTRTM ATR (Attenuated Total Reflectance) accessory, was used to record IR spectra (Derenne, Van Hemelryck, Lamoral-Theys, Kiss, & Goormaghtigh, 2013).

2.6. Statistical analysis

To verify the statistical significance, means ± SD of five independent measurements were calculated. One-way analysis on variance (ANOVA) for statistical evaluation of results was used, following by Duncan's new multiple range test to assess differences between group's means. P values of <0.05 were considered to be significant.

3. Results and discussion

3.1. Polyphenols, flavonoids, flavanols and tannins

The bioactive compounds in fresh kiwi fruit 'Hayward' smoothies and its samples prepared with different amounts of white sugar in water and methanol extracts are shown in Table 1, A, B, C. As can be seen, the contents of polyphenols in water extracts were higher than in methanol extracts. The highest content of polyphenols was found in water extract of 'Hayward0' fresh smoothie sample. In other samples the polyphenols changed during their treatment. The significantly lowest content of polyphenols was found in 'Hayward25' smoothies after storage. The contents of flavonoids, flavanols and tannins in water extracts were higher than in methanol extracts. In our previous and cited reports were determined bioactive compounds and among them flavonoids, flavanols and phenolic acids (Leontowicz et al., 2016). As can be seen, the contents of the bioactive compounds extracted with water and methanol differ significantly. Our results are in line with other reports, which discussed the influence of various solvents on the composition of extracted bioactive compounds and different extraction conditions (Dahmoune, Nayak, Moussi, Remini, & Madani, 2015; Golmohamadi, Moller, Powers, & Nindo, 2013) and showed that it was observed that total phenolics, tannins, flavonoids and antioxidant activities in microwave-assisted extracts were in higher amounts than in traditional extracts. The thermal process damages the nutritional and sensory properties of products (Sampedro et al. 2010).

Our results are in line with Iwasawa et al. (2011) that kiwi fruit is rich in polyphenols and has immunostimulatory activity. Landl, Abadias, Sarraga, Vinas, and Picouet (2010) prepared apple puree by high pressure system during 3 weeks of refrigerated storage of 5 °C, which effected ascorbic acid and total phenolic content. Our results were in line with Manea (2013), where preservation of fruit juices (elder, beetroot, red grapes) at the refrigeration temperature (at 4 °C in absence of air) was shown to conserve anthocyanins and tannins. Vitamin C felled with 91.43% in comparison with our results of about 25% and for 'Hayward10' and 'Hayward15' only from 2% to 9% (Table 1B). The anthocyanins presented a decrease of 33.12% in comparison with our results (31%, Table 1B); therefore juice registered a high stability of color after storage period. The tannin content was lower by 37.62% in comparison with our results in the range of 37–46% after the storage period. Our results are in line with Zhang and Liu (2015), following exactly the same process of preparation of juice as we prepared smoothies, except the filtration and enzyme treatment. The preparation of smoothies in this research involved selection and cleaning of fruits, preparation of fruit normal smoothies with sugar, cold enzyme killing and protecting color treatment and sterilization of final product in cold. The resulting smoothies completely preserve fresh fruits and nutritional value and health functions. The presented process is similar to Han and Lim (2014), with the exception of sterilizing following cooling, freezing, filling in the cube-shaped container, and then rapidly freezing. The consumers of such preparation drink

Table 1
(**A**) The content of total polyphenols, flavonoids, tannins, flavanols, and antioxidant capacities of kiwi fruit Hayward (fresh) and smoothie samples in water extracts (per g DW); (**B**) anthocyanins, vitamin C, chlorophylls and carotenoids in water extracts; (**C**) methanol extracts.

Indices	'Hayward0'	'Hayward10'	'Hayward15'	'Hayward20'	'Hayward25'
(**A**)					
Polyph, mgGAE	8.19 ± 0.76^{a}	7.79 ± 0.65^{a}	7.14 ± 1.23^{b}	6.17 ± 0.45^{ab}	5.07 ± 0.45^{c}
Tannins, mgCE	2.90 ± 0.28^{a}	2.76 ± 0.24^{a}	2.56 ± 0.17^{a}	2.16 ± 0.18^{ab}	1.83 ± 0.14^{b}
Flavon, mgCE	2.47 ± 0.22^{a}	2.36 ± 0.21^{a}	2.15 ± 0.18^{b}	1.81 ± 0.15^{ab}	1.53 ± 0.13^{b}
Flavan, μgCE	0.16 ± 0.04^{a}	0.15 ± 0.03^{a}	0.14 ± 0.04^{a}	0.12 ± 0.02^{ab}	0.10 ± 0.02^{b}
FRAP, μMTE	12.83 ± 1.27^{a}	12.31 ± 1.15^{a}	11.20 ± 1.07^{ab}	9.71 ± 0.95^{b}	8.13 ± 0.71^{c}
ABTS, μMTE	22.11 ± 2.22^{a}	21.13 ± 2.12^{a}	19.46 ± 1.76^{ab}	16.72 ± 1.54^{b}	13.70 ± 1.14^{c}
CUPRAC, μMTE	17.14 ± 1.64^{a}	16.22 ± 1.54^{a}	15.04 ± 1.34^{ab}	12.91 ± 1.12^{b}	10.58 ± 1.03^{c}
DPPH, μMTE	10.74 ± 0.96^{a}	10.23 ± 0.74^{a}	9.34 ± 0.66^{ab}	8.01 ± 0.48^{b}	6.63 ± 0.31^{c}
(**B**)					
Anthoc, mgCGE	20.21 ± 2.15^{a}	18.87 ± 2.54^{ab}	17.86 ± 1.76^{ab}	14.86 ± 1.76^{b}	13.87 ± 1.65^{b}
Vit C, AAmg	5.63 ± 0.51^{a}	5.51 ± 0.45^{a}	5.11 ± 0.45^{a}	4.28 ± 0.41^{b}	4.20 ± 0.37^{b}
Chlor **a**, μg	56.15 ± 5.27^{a}	54.25 ± 5.76^{ab}	55.18 ± 5.56^{a}	40.23 ± 4.34^{b}	21.15 ± 2.06^{c}
Chlor **b**, μg	15.98 ± 1.54^{a}	13.40 ± 1.34^{ab}	14.20 ± 1.41^{a}	8.25 ± 0.65^{b}	6.18 ± 0.47^{c}
Chlor **a** + **b**, μg	72.13 ± 7.43^{a}	67.65 ± 6.12^{ab}	69.38 ± 6.23^{a}	48.48 ± 4.18^{b}	27.33 ± 2.06^{c}
Xant + Car, μg	26.14 ± 2.68^{a}	23.14 ± 2.32^{ab}	24.62 ± 2.65^{a}	18.41 ± 1.31^{b}	11.18 ± 1.24^{c}
(**C**)					
Polyph, mgGAE	6.97 ± 0.58^{a}	6.57 ± 0.61^{a}	5.78 ± 0.54^{ab}	5.54 ± 0.45^{b}	3.79 ± 0.34^{c}
Tannins, mgCE	2.47 ± 0.12^{a}	2.33 ± 0.12^{a}	2.05 ± 0.12^{ab}	$1.96 \pm 0.\ 8^{b}$	1.33 ± 0.05^{c}
Flavon, mgCE	2.11 ± 0.34^{a}	1.99 ± 0.76^{a}	1.75 ± 0.25^{ab}	1.67 ± 0.11^{b}	1.14 ± 0.14^{c}
Flavan, μgCE	0.14 ± 0.07^{a}	0.13 ± 0.03^{a}	0.11 ± 0.03^{ab}	0.09 ± 0.01^{b}	0.07 ± 0.03^{c}
FRAP, μMTE	10.87 ± 1.16^{a}	10.27 ± 1.01^{a}	9.05 ± 0.94^{ab}	8.66 ± 0.71^{b}	5.94 ± 0.53^{c}
ABTS, μMTE	18.82 ± 1.75^{a}	17.71 ± 1.54^{a}	15.62 ± 1.21^{ab}	14.91 ± 1.71^{b}	10.21 ± 1.43^{c}
CUPRAC, μMTE	14.59 ± 1.45^{a}	13.75 ± 1.47^{a}	12.11 ± 1.26^{ab}	11.59 ± 1.13^{b}	7.93 ± 0.34^{c}
DPPH, μMTE	9.14 ± 1.16^{a}	8.62 ± 0.74^{ab}	7.58 ± 0.54^{b}	7.26 ± 0.76^{b}	4.97 ± 0.54^{c}

(**A**), (**B**), (**C**), Mean ± SD (standard deviation) of 5 measurements. Averages in rows marked with different letters differ significantly ($P < 0.05$). Abbreviations: ABTS, 2, 2′-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt; FRAP, Ferric-reducing/antioxidant power; DPPH, 1, 1-diphenyl-2-picrylhydrazyl; CUPRAC, Cupric reducing antioxidant capacity; Polyph, polyphenols; Flavon, flavonoids; Flavan, flavanols; CE, catechin equivalent; GAE, gallic acid equivalent; DW, dry weight; TE, Trolox equivalent; Anthoc, anthocyanins; mgCGE, cyanidin-3-glucoside equivalent; Vit C, vitamin C, AA, ascorbic acid; Chlor **a**, chlorophyll **a**; Chlor **b**, chlorophyll **b**; Xant, xanthophylls; Car, carotenes; 'Hayward0', 'Hayward10',15, 20, 25, smoothies prepared with 0%, 10%, 15%, 20%, 25% of sugar.

fruit juice or smoothies preserving the taste. Addition or mixture of different fruits increases the amount of bioactive compounds, so Bobinaite et al. (2016) showed that addition of raspberry marc extract increased total phenolic acids, anthocyanins and anti-oxidative potential in prepared purees made from different fruits. Based on the results of various antioxidant activity methods, the greatest preventative antioxidant capacity of consumed beverages in Korea was found in pomegranate as the development of healthy beverages (Kim et al., 2014).

3.2. Total antioxidant capacity

The total antioxidant capacity of studied kiwi fruit smoothies are shown in Table 1, A and B. In order to receive reliable data, the total antioxidant capacities were determined by four complementary assays: ABTS, DPPH, FRAP and CUPRAC. The obtained results by these four methods were different, but the relationship between these methods and the polyphenols was similar to our previous reports (Park et al., 2013, 2014). As can be seen, according to four assays, the significantly highest level of antioxidant capacity was registered in fresh 'Hayward' smoothie, and then slightly decreased in smoothies with addition of sugar. Such relationships are found in both aqueous and alcoholic extracts. As was shown above, these samples have also the highest content of polyphenols (Table 1A and 1B). Our results are in line with Krupa, Latocha, and Liwinska (2011) who showed a strong correlation between polyphenol contents, vitamin C and antioxidant capacities.

Keenan et al. (2010) also showed significant reductions in antioxidant activity and phenolic content at the applied pressure and a maximum treatment time of 5 min. Our results (Table 1B) are in line with Benlloch-Tinoco et al. (2015b), where microwaves and conventional heating led to marked changes in the chlorophyll (42–100% losses) and carotenoid (62–91% losses) content. The majority of carotenoids are usually ignored, using conventional scavenging assays. A main limitation is that few methods allow for the successful measurement of antioxidant activity in lipophilic fractions and most of carotenoids are found in lipophilic fraction. Since CUPRAC assay was used, which is active for both hydrophilic and lipophilic antioxidants and perfectly works in acetone solution, therefore carotenoids were included in the total antioxidant capacity. Total chlorophyll decreased during the preparation of smoothies from 6 to 62%. These results highlighted that the pigment composition of freezed kiwi fruit was more similar to that of the fresh fruit and better preserved during storage. Xanthophylls and carotenes decreased from 13.0% to 72.9%. These results showed that the pigment composition was similar to the fresh fruit with the addition of sugar of 10–15%, but then decreased drastically. The amount of anthocyanins decreased from 6.6% to 31.4% and the vitamin C from 6% to 57% (Table 1B). These results are also in agreement with George et al. (2013) that pureed fruit and vegetable products retain many beneficial components, including flavonoids, carotenoids, vitamin C. Our results are in agreement with other reports (Alvarez-Fernandez et al., 2014; Golmohamadi et al., 2013), where in preparation of the strawberry and red raspberry purees maintain the fruit's non-anthocyanin phenolic composition and in vitro antioxidant activity as determined by ORAC and DPPH methods. This fact suggests that strawberry puree could be considered a valuable ingredient for producing food derivatives. Polymerized proanthocyanidins were found to predominate in most of the products, except for the black currant smoothies, where the main group of polyphenols was anthocyanins (Teleszko & Wojdylo, 2014). Hydrostatic high-pressure application in the industrial line of nectarine puree presented some advantages compared to the thermal treatment; however, some of the changes found were lessen during the storage period (Garcia-Parra et al., 2011). It is important that the fresh product will have high

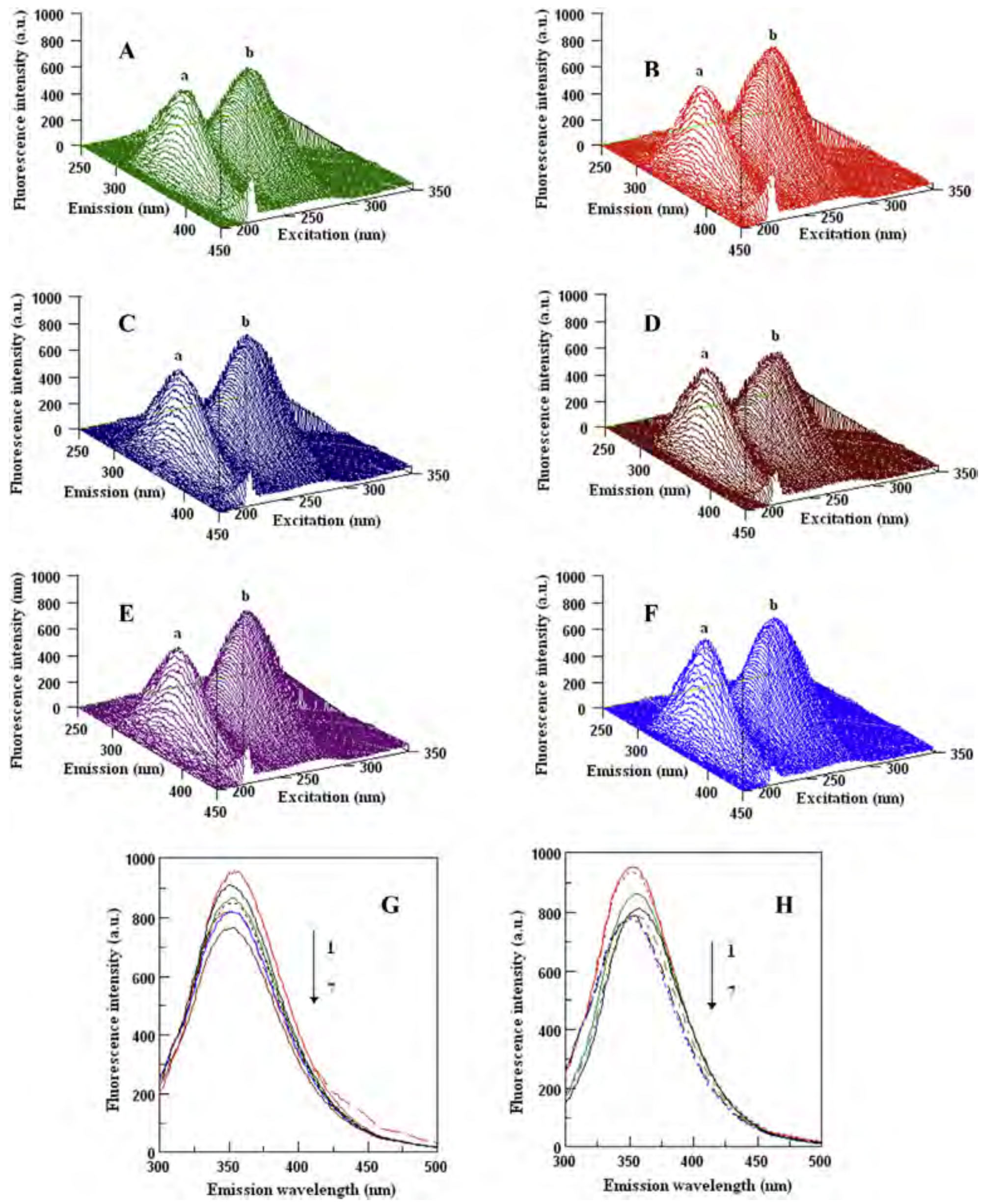

Fig. 2. 3D fluorescence spectra of methanol extracts of smoothie samples after interaction with HSA: **A, B, C, D, E, F,** 'Hayward10', HSA, 'Hayward20', 'Hayward0', 'Hayward25', 'Hayward15'. [In each sample of methanol extracts two peaks **a** and **b** are shown (Table 2A)]. 2-D emission spectra of kiwi fruit smoothies after interaction with HSA: **H**, in methanol extracts (lines 1–7, HSA in buffer; HSA in methanol; 'Hayward25', 'Hayward20', 'Hayward15' 'Hayward10', 'Hayward0', see Table 2B). **G**, in water extracts (lines 1–7, HSA in buffer; HSA in water; 'Hayward25', 'Hayward20', 'Hayward15' 'Hayward10', 'Hayward0', see Table 2C). Abbreviations: 'Hayward0', 'Hayward10', 'Hayward15', 'Hayward20', 'Hayward25', smoothies prepared with 0%, 10%, 15%, 20%, and 25% sugar after storage. Excitation wavelength scan: 200–350 nm. Emission wavelength scan: 250–450 nm.

antioxidant activity. Ledeker, Chambers, Chambers, and Adhikari (2012) showed that in the processed mango puree and subsequently mango sorbet is the most important to select appropriate cultivars what is in line with our findings. The fresh kiwi fruit obtained high bioactivity, and then the processed fruit preserved its quality. As it was shown above, our preparation involved only freezing conditions. It is opposite to Gonzalez-Cebrino, Garcia-Parra, Contador, Tabla, and Ramirez (2012) who showed that for storage stability of plum purees after high-pressure treatments heat pretreatments are required. Storage time and temperature were critical factors for peach puree phytochemical profile (Oliveira, Gomes, Alexandre, Almeida, & Pintado, 2014). According to Marszalek, Mitek, and Skapska (2015) in strawberry puree conventional thermal processing caused higher degradation of polyphenols (7%), anthocyanins (20%) and vitamin C (48%) compared to microwave heating at 120 °C. Benlloch-Tinoco, Igual, Rodrigo, and Martinez-Navarrete (2015a) showed the same evidence that the storage temperature had a significant impact on both the shelf-life and the nutritional and functional value of the kiwi fruit puree samples: the higher the temperature, the significantly faster the rate of both the sample spoilage and the loss of the bioactive compounds. A longer shelf-life (123 days at 4 °C) and preservation of bioactive compounds (57–67%) were obtained when microwave heating was the technology selected to process the kiwi fruit puree. Fernandez-Sestelo et al. (2013) also showed that high pressure processing followed by chilled storage can extend the shelf-life of kiwi puree while maintaining its overall quality. Walkling-Ribeiro, Noci, Cronin, Lyng, and Morgan (2010) proposed instead of high heat combination of moderate heat and pulsed electrical fields were used for smoothie-type beverages containing cranberry, black currant and bilberry purees.

3.3. Fluorescence and FTIR findings

2D fluorescence spectra showed the results described below. At emission of the wavelengths from 355 nm to 352 nm was recorded spectrum of methanol extracts of fresh and treated smoothies with sugar contents (0–25%). The interaction of polyphenols with HSA quenched the emission characteristics of HSA. The observed emission spectral shifts proved the effective use of fluorescent data for the determination of binding properties, using the difference of the initial and final fluorescence intensity. The interaction of polyphenols with HSA showed the decrease in the fluorescence intensity. The lowest line from the top (Fig. 2H) showed the highest binding ability of 15.8% in methanol for 'Hayward0' sample. The binding ability has decreased for 'Hayward10' and 'Hayward15' of about 35%. The binding ability (Fig. 2G, the lowest line from the top) showed the highest ability of 16.8% in water extracts. The decrease in 'Hayward10' and 'Hayward15' was about 8%. The binding abilities were in direct correlation with the polyphenols and antioxidant capacities of smoothies. The main two peaks **a** and **b** were with the highest FI (Table 2A and 2B, Fig. 2C, E, Fig. 3C, E) were in treated sample of smoothies with 20 and 25% sugar of about 723, 720 and 878 and 860 A.U. The lowest was for Hayward0 and Hayward10 (Table 2A, B, Fig. 2A, D, Fig. 3D, F with 687, 695 and 836 and 846 A.U.). The changes in the fluorescence intensity of treated and Control samples corresponded with the changes of polyphenol compounds (Table 1). The present results differ from the presented for the harvest of 2012 (Park et al. 2013, 2014). The observed peaks by 3-D fluorescence showed differences in the position of the main peaks and their fluorescence intensity for kiwi fruit treated smoothies in comparison with fresh sample. HSA shows the following shifts (cm^{-1}): 1655, 1548, 1454, 1400, 1327, 1265, 1171, 1125. The interactions of HSA with polyphenols extracted with water from smoothies were characterized by FTIR spectroscopy. There was a slight major spectral shifting for protein amide I band at 1655 cm^{-1} (mainly C=O stretch) and amide II band at 1548 cm^{-1} (C–N stretching coupled with N–H bending modes) in the presence of polyphenols (Bourassa, Kanakis, Tarantilis, Pollissiou, & Tajmir-Riahi, 2010). Changes were observed for HSA amide I band in the range of 1714 cm^{-1} and amide II band at 1593 cm^{-1} (Fig. 4). The results suggested that the increase in intensity of the amide I and II bands was due to polyphenols from polyphenols binding to the C=O, C-N, and N-H groups of protein (Dubeau, Bourassa,

Table 2
(**A**) Three-dimensional fluorescence spectral characteristics of polyphenol kiwi fruit extracts, HSA and HSA-polyphenol complex in methanol; (**B**) methanol extracts; (**C**) Two–dimensional fluorescence spectral characteristics of HSA and smoothies water extracts; (D) Matching of the peaks (%) in the FTIR spectrum of extracted with methanol phenols in interaction with HSA.

(**A**)

Samples	Peak a		Peak b		
	$\lambda_{ex}/\lambda_{em}$	FI	$\lambda_{ex}/\lambda_{em}$	FI	% binding
HSA + MeOH	227/349	766.3 ± 9.5[a]	279/352	884.2 ± 9.8[a]	
HSA + 'Hayward25'	228/350	723.4 ± 7.7[ab]	278/352	877.6 ± 9.1[a]	6.3 ± 0.6[c]
HSA + 'Hayward20'	228/345	720.3 ± 7.8[ab]	280/357	860.2 ± 9.1[a]	8.7 ± 0.7[bc]
HSA + 'Hayward15'	228/347	710.4 ± 7.9[ab]	280/350	852.2 ± 8.4[b]	10.9 ± 1.1[b]
HSA + 'Hayward10'	228/350	695.1 ± 8.5[b]	278/352	846.3 ± 9.1[a]	13.6 ± 1.3[ab]
HSA + 'Hayward0'	227/349	686.8 ± 9.5[b]	279/352	835.6 ± 9.8[b]	17.4 ± 1.5[a]

(**B**)

Number of lines	Samples	λ_{em}	FI	% binding
1	HSA + buffer	355	953.2 ± 15.8[a]	–
2	HSA + MeOH	350	911.8 ± 12.7[a]	4.3 ± 0.46[c]
3	HSA + 'Hayward25'	352	867.0 ± 11.4[b]	4.9 ± 0.4[c]
4	HSA + 'Hayward20'	353	850.4 ± 10.5[b]	6.7 ± 0.5[bc]
5	HSA + 'Hayward15'	353	820.8 ± 9.6[bc]	10.0 ± 1.1[b]
6	HSA + 'Hayward10'	353	819.2 ± 9.26[bc]	10.2 ± 1.2[b]
7	HSA + 'Hayward0'	353	767.5 ± 8.5[c]	15.8 ± 1.66[a]

(**C**)

Number of lines	Samples	λ_{em}	FI	% binding
1	HSA + buffer	355	953.2 ± 15.8[a]	–
2	HSA + Water	350	934.5 ± 14.2[a]	2.0 ± 0.2 [c]
3	HSA + 'Hayward25'	356	863.2 ± 12.6[b]	7.6 ± 0.8[b]
4	HSA + 'Hayward20'	356	812.9 ± 9.9[bc]	13.0 ± 1.2[ab]
5	HSA + 'Hayward15'	355	789.5 ± 12.4[c]	15.5 ± 1.4[a]
6	HSA + 'Hayward10'	355	786.2 ± 9.6[c]	15.9 ± 1.6[a]
7	HSA + 'Hayward0'	352	777.1 ± 7.9[c]	16.8 ± 1.8[a]

(**D**)

Range of bands	1800–750 cm^{-1}			
Samples	Matching of sample/sample/HSA (%)			
	'Hay0'+HSA	'Hay10'+HSA	'Hay15'+HSA	'Hay20'+HSA
Hay0 + HSA	99.87 ± 9.14[a]	89.67 ± 9.06[a]	83.86 ± 8.25[ab]	69.18 ± 6.85[b]
HSA	6.61 ± 0.64[c]	11.32 ± 1.14[b]	11.59 ± 1.09[b]	31.81 ± 3.24[ab]

(A), (B), (C), (D), Mean ± SD (standard deviation) of 5 measurements. Average in columns marked with different letters differ significantly ($P < 0.05$). Abbreviations: Hayward0, Hayward10, 15, 20, 25, smoothies, prepared with 0%, 10%, 15%, 20%, 25% of sugar; HSA, human serum albumin; λ_{em}, λ_{ex}, wavelength of emission, excitation in nm; FI, fluorescence intensity in arbitral units; (D), Hay, Hayward.

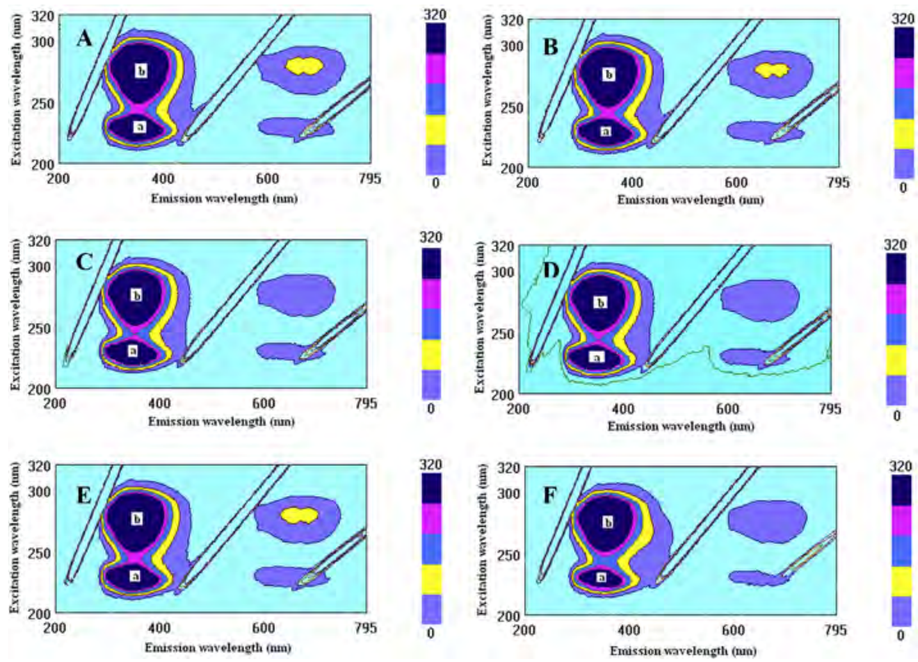

Fig. 3. Corresponding contour maps of three-dimensional fluorescence spectra of different extracts of smoothie samples: **A, B, C, D, E, F,** HSA, 'Hayward15', 'Hayward20', 'Hayward0', 'Hayward25', 'Hayward10', see Table 2A, B). Abbreviations: 'Hayward0', 'Hyward10', 'Hayward15', 'Hayward20', 'Hayward25', smoothies prepared with 0, 10, 15, 20, 25% of sugar after storage. HSA, Human Serum Albumin; Fluorescence intensity, arbitral units; Excitation wavelength scan: 200–320 nm; emission wavelength scan: 200–795 nm. In each sample of methanol extracts several peaks are shown (see Table 2A for corresponding peaks). HSA 2 mM × 10^{-6}, smoothie extracts in methanol 100 μl from 25 mg/ml (0.83 mg/ml); λex 280 nm and λem 300 nm; the reaction was during 1 h at room temperature. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

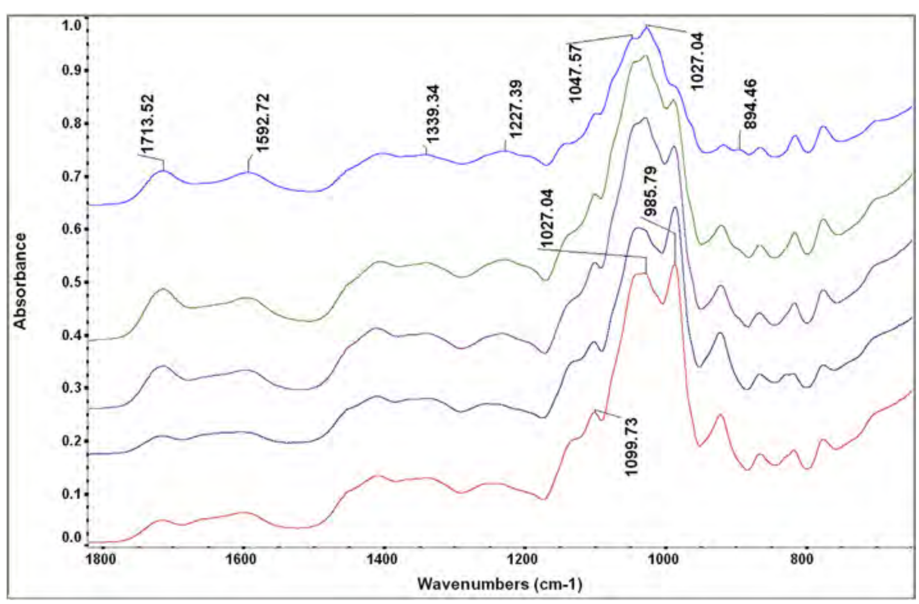

Fig. 4. FTIR spectra of water extracts of smoothies samples after interaction with HSA: **a**, **b**, **c**, **d**, **e**, 'Hayward25', 'Hayward20', 'Hayward15', 'Hayward10', 'Hayward'. A Nicolet iS 10 FT-IR Spectrometer with the smart iTRTM ATR (attenuated total reflectance) accessory was used to record IR spectra.

Thomas, & Tajmir-Riahi, 2010). The best matching of peaks was with 'Hayward10' because the changes in the protein structure was similar, but starting from 'Hayward20' was lower, then the decrease in the ability of polyphenols and other compounds was about 37%.

4. Conclusions

It is known that kiwi fruit possess high antioxidant activity and can prevent heart diseases, therefore the aim of the present research was to preserve the antioxidant status of processed kiwi fruit product. Kiwi fruit smoothies with addition of sugar were prepared and stored under freezing conditions. The quality of the processed product was compared with fresh fruit. The bioactive compounds (ascorbic acid, total phenols, anthocyanins, chlorophylls, carotenoids, tannins, flavonols and flavonoids) were determined before and after treatment and relative high presentation of bioactivity by freezing was found. Three different assays were used for determination of antioxidant activity: $ABTS^+$, FRAP, DPPH, and CUPRAC. 3-D fluorescence and FTIR were employed in order to compare the bioactivity of smoothies after treatment with conventional and advanced analytical methods. The best results were obtained in smoothies with 10 and 15% added sugar. These products are available source for natural antioxidants.

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